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Opening Lecture

Sustainable Agriculture – A Logical Approach to Ecological Agriculture

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Since the middle of the 20th Century, modern agriculture has been a remarkable success. However, it is widely accepted that the future of modern agriculture is threatened by a diminishing resource base due to depletion and contamination. This situation has led to a reassessment of current agricultural practices and to the development of new approaches, one of which is sustainable agriculture.

Sustainable agriculture is defined as an integrated system of plant and animal production aimed at satisfying human food and fiber needs over the long term, while making efficient use of nonrenewable resources, maintaining environmental quality and sustaining the economic viability of farm operations. This approach combines modern agricultural practices with some principles of "alternative" agriculture approaches, such as organic agriculture. Sustainable agriculture is not a completely defined and applicable method. Rather, it is a goal in the development of new and better agricultural practices.

The major components of sustainable agriculture include: crop rotation; minimum tillage; pest control strategies that are not harmful to natural systems, farmers or consumers; reduced use, but not elimination, of chemicals, and the use of organic matter for soil amelioration. Special emphasis is placed on the integration of knowledge from various disciplines and on the adaptation of the agricultural practices to specific crops, local conditions and populations.

It seems that agriculture worldwide is in an accelerated transition period, the long-term goal of which is sustainable agriculture. *(L)**

*L = lecture sessions; P = poster (market place) sessions.

Integrated Management of *Alternaria solani* in Potatoes

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A model of integrated control of early blight in potatoes caused by *Alternaria solani* was developed and verified in research carried out during 1992–94. Several assumptions were considered while setting up the model: (i) systemic fungicides are more effective than protectant fungicides; (ii) the sensitivity of the host to the pathogen decreases as the season progresses; and (iii) it is essential to limit the use of systemic fungicides in order to reduce the risk of development of resistance. The model integrates plant age resistance and the efficiency of fungicides used. Basic rules for timing application of sprays in the integrated control model are: (i) start chemical sprays at tuber initiation or, at the latest, when the first lesions appear; (ii) apply sprays at long intervals at the beginning of the spraying season and more frequently toward the end of the season; and (iii) use the protectant fungicides initially and systemic fungicides later in the season.

The model assumptions were tested in field trials. The contribution of systemic fungicides was greatest when they were sprayed toward the end of the season. The possibility of compensating for change in age-related resistance of the host by adjusting spray timing and frequency was also examined. The integrated control scheme included protectant sprays at increasing frequency, starting with the disease outbreak. As the season progressed, along with increasing host sensitivity, a systemic fungicide was used with increasing frequency as well. The integrated control plan was effective in reducing disease severity and increasing yields in all the potato trials. It was at least as effective as treatments which included routine sprays with protectant or systemic fungicides; the total number of sprays was lower when the integrated control plan was used. (*L*)

Degeneration Syndrome of *Anemone* Plants and Its Control by Soil Solarization

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The phenomenon of degeneration of *Anemone* plants has been known in Israel since the late 1960s. The characteristics of the syndrome are leaf yellowing, brown lesions on the lower parts of the petioles, and root rot that leads to uprooting of the plants from the soil. In affected fields, flower yield and quality (as expressed by stem length) are reduced; the flowers are not suitable for export. First symptoms are visible shortly after the first rain, at about the middle of November. The degeneration syndrome is very common in flooded or very wet soils and in monoculture systems, even in the second cropping year. It was evident even when the field had been left for 10 years without growing *Anemone* plants. The cause of the syndrome is still unknown, but very likely it is soilborne.

In experiments that were carried out for 4 years in fields with a history of the syndrome, it was demonstrated that soil solarization is very effective in controlling the syndrome. Solarization

increased the yield of flowers by 72–117% in comparison with the untreated control. The quality of flowers, as expressed in length of flower stems, was also improved, to the level of export quality. Weed control was an additional benefit of solarization. Soil solarization has been adopted by most growers of this crop. (*P*)

The Use of UV-Absorbing Films for Protection of Different Crops against Virus Diseases Vectored by *Bemisia tabaci*

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Field observations carried out during the summer of 1993 indicated that cucumbers and tomatoes grown in high tunnels covered with a particular polyethylene (PE) film (IR Veradim, Ginnegar Plastic Products, Kibbutz Ginnegar, Israel) were well protected against cucurbit yellowing virus (CYV) and tomato yellow leaf curl virus (TYLCV), both of which are vectored by *Bemisia tabaci* Gennadius. Almost 100% disease incidence was recorded in neighboring tunnels covered with regular PE sheets (IR Nectarine, Ginnegar Plastic Products).

In the autumn of 1994 a field experiment was carried out in the Besor area of Israel, where the whitefly population has reached high levels and TYLCV incidence is very heavy. Tomatoes (*Lycopersicon esculentum* var. 144) were grown in high tunnels (6x6x2.7 m) covered with different plastic films. Plants were sprayed once a week with an anti-whitefly insecticide. Two months after transplantation the average TYLCV disease incidence under IR Veradim, Solarig (Palrig, Kibbutz Ne'ot Mordekhai) and Rav-Hozek (Erez Thermoplastic Products, Kibbutz Erez) was 50%, 30% and 20%, respectively, compared with 93% under the regular PE sheets. The protection conferred by IR Veradim resulted also in a significant delay in plant infection and consequently led to a reduction in symptom severity. Monitoring the whitefly population with yellow sticky traps indicated a significantly lower number of whiteflies in tunnels covered with IR Veradim. It is assumed that elimination of UV from the light spectrum by UV-absorbing plastic films interferes with the ability of the insects to identify the covered tunnels during landing and also affects the inoculation efficiency of insects that have already invaded the covered structures. (*L*)

Effect of the Biocontrol Agent *Trichoderma harzianum* T-39 on the Pathogenicity of *Botrytis cinerea*

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The biocontrol agent *Trichoderma harzianum* T-39 controls grey mold disease caused by *Botrytis cinerea*. Disease reduction has been achieved under commercial conditions in vineyards and in protected tomato and cucumber crops. Several mechanisms have been suggested for the control achieved by different *Trichoderma* isolates, including: mycoparasitism, production of inhibitory substances, competition and induced resistance. We have examined the effect of strain T-39 on two components of the pathogenicity process of *B. cinerea*: the germination of conidia and the activity of pectolytic enzymes produced by *B. cinerea*. The germination of *B. cinerea* conidia and the length of germ tubes were determined in the presence or absence of *T. harzianum*

strain T-39. The biomass of the pathogen germ tubes on bean leaves was estimated (% germinating conidia x average length of germ tubes). Twenty hours after inoculation on bean leaves, the germ tubes' biomass was reduced by the biocontrol agent. However, no difference in the amount of mycelium was detected 48 h after inoculation. The pectolytic enzymes polygalacturonase, pectin methyl esterase and pectate lyase produced by *B. cinerea* are important in causing tissue breakdown. The activity of these enzymes produced by *B. cinerea* on bean leaves, alone or in the presence of *T. harzianum* T-39, was investigated. In the presence of T-39 the activity of all three enzymes was reduced. The reduction in enzyme activity was detected even 4 days after inoculation. Disease reduction was achieved whenever the activity of the pectolytic enzymes was reduced. It is suggested that the effect of *T. harzianum* on these enzymes is part of the mechanism by which the biocontrol agent exerts disease control. (*L*)

Control of Grape Fungal Rot in Vineyards

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Zohar LQ-215 is a non-toxic liquid detergent, developed and manufactured by Zohar Detergent Factory, in Kibbutz Dalia, Israel. It is harmless to human beings and warm-blooded animals and biodegradable, with no negative impact on the environment. The product is registered in Israel as an insecticide against the young instars of the whitefly *Bemisia tabaci*. Zohar LQ-215 is also effective in preventing infection by nonpersistent viruses vectored by aphids.

During the past 3 years many tests have been performed with Zohar LQ-215 as a fungicide against common fungal diseases such as powdery mildew and grape bunch rot. Our latest experiments were performed in Moshav Amazya in the Lakhish region (in the south of the country) during 1993 and 1994 on 'Thompson' (Sultanina) grapes. The fungi *Aspergillus* spp. and *Rhizopus* spp. were found to be the cause of the grape rot in this area

In these experiments, 0.4% of Zohar LQ-215 was sufficient to reduce successfully the number of rotten bunches. Moreover, it decreased the degree of infection of the bunches. It proved to be better than conventional chemical products in use. No phytotoxic effects were found on the bunches during the treatments and the shelf life and quality of the grapes were not affected [Dubitzki *et al., Hassadeh* 74:417-418 (1994)]. (*P*)

Effects of Sublethal Concentrations of Triadimefon on Rust Development in Fast- and Slow-Rusting Barley Lines

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Barley (*Hordeum vulgare* L.) is the world's fourth most important cereal crop, resistant to drought and salinity, and yielding grain even on the fringe of the desert.

One of the major pathogens of barley is *Puccinia hordei* Otth, which occurs wherever barley is grown. Clifford (1985) distinguished two main types of resistance to the disease. Type I resistance is known as low-reaction resistance; it is expressed as a hypersensitive reaction associated with necrotic lesions. Type II resistance is referred to as 'partial' resistance or slow rusting. The uredia formed are mainly of infection type 3 and low to moderate infection severity.

In the present study, by using sublethal concentrations of Bayleton (triadimefon) WP50 before inoculation at the seedling or adult plant stage with *P. hordei*, the host responses evoked were similar to those characterizing slow rusting. By applying 30 mg/*l* triadimefon to the susceptible line 'Nigrate', receptivity was reduced by 40.0%, sporulation was decreased by 45.9%, and the latent period of infection was prolonged by 2.2 days as compared with the untreated control. Our conclusion is that fast-rusting lines responded to a low fungicide dosage by developing symptoms similar to those in untreated slow-rusting lines. (*P*)

Efficacy of Foliar Sprays of Phosphates in Controlling Powdery Mildews in Field-Grown Nectarine and Apple Trees

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Foliar sprays of 0.4–1.1% solutions of KH_2PO_4 (plus KOH, to establish a pH of 8–9; plus Triton X-100) and commercial systemic fungicides inhibited development of powdery mildew fungi on fruits and leaves of field-grown nectarine (cv. 'Fantasia') and apple (cvs. 'Gala' and 'Jonathan') trees. The systemic fungicides myclobutanil (Systhane 12E) and penconazole (Ophir) were more effective in controlling the disease on fruits and leaves of nectarine and apple, respectively, than was the phosphate. Alternating treatments of phosphate salt with the systemic fungicide, however, in both the 1993 and the 1994 season, enhanced the inhibitory effect against the fungus in nectarine; the effect was similar to that of the commercial treatment with systemic fungicides. Similarly, leaves of apple trees treated with systemic fungicides or with the alternating treatments of phosphate with an appropriate fungicide were the most protected against the mildew. Phosphate solutions were not phytotoxic to plant tissue. Fruits harvested from nectarine trees sprayed with phosphate alone or in alternation with a fungicide were similar in size distribution to those harvested from the commercial fungicide-based treatment, and larger than those obtained from control (untreated) trees.

The inhibitory effectiveness of phosphate salts makes them useful 'biocompatible' fungicides for application in the field for disease control. (*L*)

Hydrogen Peroxide for Control of Botrytis Blight in Cut Rose Flowers

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Botrytis blight, caused by *Botrytis cinerea*, is a widespread problem in cut rose flowers. Current methods of disease control are unsatisfactory and Botrytis blight is the major reason for discarding flowers before export. In this work we studied the possibility of controlling *B. cinerea* on cut rose flowers (cv. 'Mercedes') by means of hydrogen peroxide (H_2O_2). The chemical was applied as droplets (50 µl) at concentrations of 0.001–0.04 mM. Flowers were inoculated with *B. cinerea* using 50 µl droplets of spore suspension (10^4 spores/ml). Rose Botrytis blight was significantly suppressed (50–100% inhibition) in petals treated with 0.02 and 0.04 mM H_2O_2. Germination and germ tube elongation of conidia on rose petals was inhibited up to 90% by 0.04 mM of H_2O_2; lower concentrations of H_2O_2 resulted in partial inhibition. Treatment with H_2O_2

was effective at temperatures ranging from 4 to 25°C. The effect of H_2O_2 on disease development was also investigated in intact flowers, 2 days after harvest, by spraying the flowers with a 0.1 mM solution of H_2O_2 followed by inoculation with a conidial suspension (10^4 conidia/ml). A concentration of 0.1 mM H_2O_2 caused a 70% decrease in disease severity. Spraying 1 mM H_2O_2 was found to be toxic to the petals. Treatment by evaporating a 1.5% solution of H_2O_2 in a closed environment reduced the disease by 60%. Suppression of the disease was also observed in a shipment simulation. A method for detecting the presence of H_2O_2 on petals was developed. H_2O_2 was still present in droplets one week after application but was not detected on intact flowers that had been sprayed with a solution containing H_2O_2. *(P)*

Examination of a New Approach for Control of Calyx End Rot of Date Palm

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Calyx end rot, incited by *Aspergillus niger* and *A. phoenicis*, is a common disease in all date-growing areas around the world. The disease causes heavy losses to the Deglet Noor and Medjool varieties in Israel. Control experiments, based on spraying bunches with various fungicides, did not provide effective control. Analysis of the results obtained from artificial inoculations of detached fruits indicated that the chances for effective control using fungicide sprays are very poor. An alternative control strategy, aimed at reducing the inoculum potential in the grove, was explored. The results of numerous tests showed that the atmosphere in date groves is saturated with *Aspergillus* spores, and that the nets used as bunch covers and the fruit rind of healthy fruits are highly contaminated with spores. The soil under the trees was found to contain enormous quantities of spores. The surface and upper layers of the soil contained many fruits at various stages of disintegration and full of spores. This inoculum was concentrated mainly around the base of trees and near the drip irrigation system. Application of formalin (formaldehyde solution), a general biocide, to naturally and artificially contaminated soils, completely eradicated the fungus. By controlled application of formalin the pathogen could be eradicated to the desired depth (4 cm). Various aspects of the utilization of formalin applications in date groves were discussed. *(L)*

Application of Cross Protection in Cucurbits against Zucchini Yellow Mosaic Virus in Israel

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Zucchini yellow mosaic virus (ZYMV) is frequent in many parts of the world, Israel included. It causes severe epidemics which result in many cases in a total loss of the crop. The fruit is stunted, twisted or deformed and unmarketable. The virus exhibits typical potyvirus characteristics. The most economic way to avoid virus epidemics is by using resistant cultivars. However, there are no available sources for resistance against this virus. Other modes of control include sanitation, use of virus-free seeds, and application of mineral oils alone or with the addition of pyrethroids. These methods greatly reduce losses due to the virus but do not prevent them when the inoculation pressure is high. In the present work, we demonstrated the application of cross protection using the

French mild viruses isolate ZYMV-WK, that was kindly provided by Dr. H. Lecoq from the Department of Plant Pathology, INRA, Montfavet, France. This virus isolate is poorly aphid transmissible and was found stable in other cross protection experiments that were carried out until now in various parts of the world. Cross-protected squash at Sha'ar HaNegev yielded up to 30% more than the nonprotected control. The fruit was of better quality and marketable. A method that will allow mass inoculation of cucurbit seedlings is now being developed. Large pilot experiments of cross protection in other parts of Israel are in progress. (*L*)

B: RESISTANCE OF PLANTS TO PATHOGENS; PHYTOALEXINS

Identification of Resistant Germplasm of *Ocimum basilicum* against *Fusarium oxysporum*

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Sweet basil (*Ocimum basilicum* L.) is the leading cultivated fresh herb grown commercially in Israel. Fusarium wilt caused by *Fusarium oxysporum* poses a severe problem for this crop. The expansion of cultivated areas of basil in Israel is accompanied by a continuous exacerbation of the disease, which causes stunting of the plants, browning of vascular tissue, dark longitudinal streaks on stems, severe wilting and defoliation. Several isolates of *F. oxysporum*, originating from roots and stems of diseased basil plants, were pathogenic on basil in growth chamber and greenhouse tests. Two stem-originated isolates of *F. basilicum* were used for further inoculations in additional tests for selection of resistant cultivars.

Resistant germplasm was identified in several basil plants of a local cultivar which was introduced from the USA and adjusted at Newe Ya'ar for local environmental and agronomic needs. Seeds were planted in the greenhouse on naturally highly infested soil. Symptomless plants were selected for self-breeding as a source for seeds of resistant germplasm, which were identified by artificial inoculations with both isolates of the pathogen. Further selection tests to improve resistance were conducted in the greenhouse on infested soil up to F_4. All individuals of the present genetic line were symptomless, whereas all individual plants of the susceptible cultivar defoliated 3 weeks after planting in infested soil. As this genetic line is demonstrating remarkable resistance against *F. oxysporum*, further crosses will be made to introduce this resistance into other germplasm of sweet basil of high commercial and agronomic value. (*L*)

Downy Mildew of Sunflower in Israel

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Sunflower plants infected with downy mildew caused by *Plasmopara halstedii* were first observed in Israel in the early 1970s (R. Kenneth, personal commun.). In 1989 and again in 1994 outbreaks of the disease on sunflower cultivar D.I.-3 were seen in the Lakhish region. Diseased plants were severely stunted and had typical systemic symptoms with heavy sporangial sporulation. On the basis of results of inoculation of differential cultivars, we determined that the fungus belongs to race 1. The downy mildew pathogen of sunflower is seed-, soil- and air-borne. Initial

infections result normally from oospores in the soil which cause systemic infection in young seedlings. Sporangia which develop on such plants may infect the apical buds of young plants, which will lead to more systemically infected plants or, if plants are older, symptomless infected plants, which may produce infected seeds. Infected seeds, if they germinate, may produce infected plants at a very low frequency or, if they do not germinate, serve as inoculum in the soil, which attacks the root system of actively growing plants. Seed treatment with metalaxyl may be effective in controlling the disease. β-aminobutyric acid, applied as a soil drench or foliar spray, also controls the disease.

Genes for resistance are available, especially in oil cultivars. Care should be taken to avoid import of infected seed. Infection in seed can be detected by microscopical techniques or by growing the seed for at least two seasons under quarantine conditions. (*L*)

Preformed Antifungal Materials and Resistance of Citrus Fruits against Pathogens

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The flavedo (exocarp) of non-infected citrus fruit contains a number of compounds possessing antifungal activity. These compounds are suggested to constitute a first line of the fruit's defense against pathogens and are referred to as 'preformed antifungal materials' (PAMs), in contrast to induced materials (phytoalexins), appearing only after pathogen attack or other stress. The PAMs identified so far in this laboratory in various citrus fruits (pomelo, lemon, lime) are terpenoids or phenolic compounds (*e.g.* coumarins and furanocoumarins). Regulating the PAM level may enable new approaches to fruit decay control.

In lemon, one of the important PAMs was identified as the monoterpene aldehyde citral. During fruit senescence on the tree or in storage, the citral content in lemon flavedo declined in parallel with the increase in fruit susceptibility to decay and with the increase in the monoterpene ester neryl acetate. Neryl acetate exerted practically no inhibitory activity against the citrus postharvest pathogen, *Penicillium digitatum* Sacc., and, in concentrations below 500 ppm, even stimulated development of the pathogen. Prevention of citral degradation in lemon by postharvest application of gibberellin, 2,4-D or heat treatment, was correlated with enhancement of fruit disease resistance as compared with untreated fruit.

Isolation and identification of PAMs from other citrus fruits, such as grapefruit and orange, are currently in progress. (*L*)

Columbianetin, a New Phytoalexin Associated with Celery Resistance to Pathogens during Storage

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Columbianetin was found to be a new phytoalexin associated with celery (*Apium graveolens* L.) resistance to pathogens during storage. *In vitro*, columbianetin had at least 80 times greater antifungal activity than furanocoumarins (psoralens and angelicin). *In vivo*, the concentration of furanocoumarins in celery was 8 μg/g f.wt. (fresh weight), which is less than 0.25% of the concentration required for growth inhibition of celery pathogens. However, the concentration of

columbianetin *in vivo* was 38 µg/g f.wt., which is close to the concentration required for growth inhibition of celery pathogens *in vitro*.

Increased susceptibility of celery to pathogens was accompanied by a decrease in columbianetin concentration and a corresponding increase in furanocoumarin concentration. After one month of storage at 2°C the concentration of total furanocoumarins increased from 8 to 85 µg/g f.wt, whereas the concentration of columbianetin decreased, under these storage conditions, from 38 to 16 µg/g f.wt. Concomitantly, the incidence of decay increased from 0 to 31%.

When celery was inoculated with the fungus *Botrytis cinerea* Pers., the concentration of columbianetin increased during the first 5 days and then started to decline. Such a pattern of accumulation and degradation is typical of phytoalexins in plants. (*L*)

C. SOILBORNE DISEASES AND THEIR CONTROL

Root Diseases of *Asclepias tuberosa*

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Asclepias tuberosa is grown in greenhouses on an area of 10 ha in the sandy soils of the Besor region. Cut flowers are in great demand in Europe, and Israel is the exclusive supplier in winter, due to the advantage of high light intensity for growing flowers. The crop, which is perennial, is planted in methyl bromide-fumigated plots, under illumination and night heating. Propagation is done through true seeds or root segments. Plants originating from seeds are genetically heterogenic, which leads to considerable variability in flower color, structure of the inflorescence and leaf shape; therefore, planting root segments may be the better alternative for a uniform crop.

Recently, severe brownish-black rots were detected in the storage root. The lesions are irregular and spread over the entire length of the root, frequently being found in the upper part of the root. This situation leads to poor sprouting, plant wilt and a reduced number of flowers per plant. Several organisms have been isolated, among which *Rhizoctonia solani, Pythium* spp. and *Erwinia* spp. were predominant.

During 1994 we investigated disease infection in two imported seed lots and ten local lots. One of the imported lots was infected with *R. solani* at a level of 6.4%, whereas all the local seed lots were free of the pathogen. Seed treatments (by dusting or dipping) with fungicides such as Monceren-T (Monceren (pencycuron) [15%] + thiram [32%]), Monceren Combi (Monceren [20%] + captan [50%]), captan and Rizolex (toclofos-methyl) reduced the disease level, whereas Kocide (copper hydroxide) and Terraclor (quintozene) had little effect; there was no significant difference between application methods. Root segments with typical lesions were dusted or dipped in one of several fungicides and planted in methyl bromide-fumigated soil at Besor. Plant development and disease expression were monitored. Generally, *R. solani* caused higher plant mortality than did *Pythium*. Rizolex, Monceren-T and Monceren Combi reduced the plant mortality rate. In addition, these treatments and others such as Dynone (prothiocarb) and Monceren (pencycuron), improved stem development.

Use of pathogen-free or treated seeds or root segments, might prevent or delay plant wilt and root rot caused by *R. solani* and *Pythium* spp. Control of these pathogens would improve the yield and quality of flowers. (*L*)

Yield Decline and Its Control in Cucumbers for Industry Grown under Monoculture

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Cucumbers for industry are grown in northern Israel in the open field, one or two crops per year. In many fields, cucumbers are grown continuously for 10–20 years; this leads to problems which are typical of monoculture systems. Yield decline is common in those fields. This phenomenon is similar to the 'soil sickness' syndrome in other crops, such as muskmelon. In muskmelons yield decline was associated with a heavy colonization of the roots by the fungi *Pythium* and *Olpidium*.

Soil disinfestation treatments (methyl bromide, formalin or soil solarization) of soils that had served for many years for cucumber growing in monoculture, improved the development of cucumber plants that were planted after the treatment (increased growth of shoots, roots and increase of leaf area); the yield was increased by 11–24%. Root colonization by *Pythium* after 30 days' growth in the untreated plots was high (75%) as compared with zero colonization in the disinfested plots. *Olpidium* was not observed in roots of cucumber plants grown in heavy loam, but was recorded in those grown in monoculture in an artificial growth medium (tuff, which is volcanic ash) system, in the coastal plain.

The phenomenon of soil sickness was reproduced in the greenhouse by planting cucumber seedlings in soil that had been collected from fields; growth retardation was evident in samples of control (nondisinfested) soil. Symptom severity was correlated with *Pythium* infection of the roots. Growth of plants in disinfested soils was improved. It is concluded that soil disinfestation is a potential tool to eliminate the soil sickness of cucumbers. The authors believe that crop rotation may further improve the results. (*L*)

Control of Soilborne Pathogens with Methyl Bromide in Controlled-Environment Systems

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The need to reduce the dosage of methyl bromide (MB) for soil fumigation requires studying various aspects of its effect on target pests or diseases. Toxicity of a pesticide is a function of its concentration (C) and exposure time (T). It is accepted that similar CxT products indicate similar levels of pathogen control. Therefore, reducing toxicant concentration by half, while doubling exposure time, will yield a level of pathogen killing which is similar to that obtained with the standard concentration and exposure time. A controlled-environment set-up to test pathogen control at different CxT products of MB was developed. The system consists of sealed glass jars which are connected to a system of nylon tubing. The sealed system and the tubing enable injection and circulation of the gas, and regulation of its concentration. Propagules of the tested

pathogen which are prepared according to test requirements and are put inside a nylon net bag, are placed inside the jar, and are exposed to MB at a controlled temperature.

The level of killing of sclerotia of *Sclerotium rolfsii* resembles that obtained with similar CxT products, but with different combinations of C and T. This resulted at high concentrations of MB and at an exposure time of less than 24 h. Higher CxT values were required to obtain the same level of killing when lower concentrations were used; this was expressed by a required exposure period longer than that calculated from the product. Effective killing was obtained only as long as the MB concentration exceeded a critical threshold. Sublethal heating, combined with a reduced dosage of MB, can improve pathogen control. This was verified by first weakening the pathogen by sublethal heating, and afterwards applying MB. A practical application of this approach is the use of MB at reduced dosage, combined with solarization. The controlled-environment system described makes it possible to test the effectiveness of fumigants under different conditions. (*L*)

Control of Verticillium Wilt with Methyl Bromide at Reduced Dosage

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Soil fumigation with methyl bromide (MB) is a common practice for controlling Verticillium wilt in many crops. However, there is a need to develop a method for reducing MB dosage. The commonly used films for MB fumigation, *e.g.* low density polyethylene (LDPE), provide a poor barrier, and enable the emission of MB into the atmosphere during fumigation. Recent studies have shown that virtually impermeable films (VIF) minimize this emission during the exposure period and extend the time MB is retained in the soil.

The effectiveness of VIF with reduced dosages of MB in the control of Verticillium wilt was examined in commercial potato fields naturally infested with the pathogen. Microsclerotia of *Verticillium dahliae* were controlled effectively to a depth of 40 cm by fumigation with MB at a rate of 50 g/m^2 using LDPE, or 25 g/m^2 using VIF. Control of *V. dahliae* microsclerotia by MB 25 g/m^2 under LDPE was again less effective. Similarly, Verticillium wilt of potato was controlled effectively by fumigation with MB at a rate of 50 g/m^2 under LDPE or 25 g/m^2 under VIF, whereas fumigation with 25 g/m^2 under LDPE was again less effective. Plants in the nonfumigated plots and in those fumigated with MB at a rate of 25 g/m^2 under LDPE collapsed 4 weeks before harvest. In contrast, plants grown in the plots that were fumigated at full dosage and those fumigated at half rate under VIF did not collapse before harvest. Subsequently, potato yield in the plots fumigated at half dosage with VIF was similar to that obtained in the plots fumigated at full dosage, and was 31% higher than in the nonfumigated plots. The percentage of big tubers (> 45 g) was 50–60% in the fumigated plots compared with 30% in the nonfumigated control plots; thus, Verticillium wilt also reduces commercial tuber quality. Effective control of *V. dahliae* and yield increase by MB at reduced dosage under VIF was observed also in a second, consecutive potato crop. Similar results were obtained in a large-scale field plot in a commercial application, demonstrating the feasibility of reducing the dosage of MB by employing VIF. (*L*)

Methyl Bromide for Soil Fumigation Towards the Year 2000

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Reducing the dosage of methyl bromide (MB) used in soil fumigation is an environmentally desirable goal. Current research is focused on using reduced dosages of MB with virtually impermeable films (VIF), and by combination with other disinfestation methods such as solarization. VIF can retain effective concentrations of the gas in the soil for longer exposure periods, and enable effective control of soilborne pests with reduced dosages. The CxT products (Concentration of MB x Time of exposure) which were recorded in soil under field conditions with reduced dosages of MB combined with VIF were similar to those with MB at full dosage using low density polyethylene (LDPE). Fumigation at reduced dosage and VIF was effective in control of several root diseases (*e.g.* Fusarium crown rot of tomato, sudden wilt of melons, Verticillium wilt of potatoes) in field experiments. The longer exposure period of MB in soil brought about by VIF results in many cases in enhanced degradation to bromide salts and, consequently, in further reduction in the emission to the atmosphere.

Escape of MB through the plastic edges is an important factor in its emission to the atmosphere. The rate of escape through the film edge is highly significant in strip fumigation, which is very common in Israel. Fumigation in wider strips and burial of the film edges to a greater depth, minimize significantly the escape of the gas from the edges.

Combining MB at reduced dosages with other methods of control, such as sublethal heating and solarization, is another means to reduce the dosage of MB. Combination of MB at reduced dosage with solarization gave effective control of Fusarium crown rot of tomato and of sudden wilt of melons. The use of impermeable films for MB fumigation, and combination with other control methods, can be effective for soil fumigation and enable its use in future with minimal environmental hazards. (*L*)

D: BIOLOGY AND EPIDEMIOLOGY

Wheat Rust Survey in Israel in 1994

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Leaf, stem and stripe rusts cause damage to wheat crops worldwide. Leaf rust caused by *Puccinia recondita* f.sp. *tritici* is the most prevalent rust disease in Israel. Outbreaks of yellow rust epidemics (*P. striiformis*) in Israel are sporadic, but when occurring they may damage wheat

severely. Stem rust (*P. graminis tritici*) appears at isolated locations on highly susceptible, late wheat cultivars.

Breeding for resistance is ecologically and economically the single, outstanding disease control measure. A wise and well-planned breeding program for resistances requires knowledge of the patterns of virulence in the rust populations, monitoring of changes in virulence type and spectrum, and choosing cultures suitable for selection of wheat breeding lines and future cultivars.

Leaf rust – In 1994 leaf rust developed in several areas of Israel on susceptible bread and durum wheats and wild emmer (*Triticum dicoccoides*). Sixty cultures were sampled and identified on 17 differential varieties carrying single genes for resistance on Thatcher background. Five UN races were identified: UN1, UN5, UN6, UN10 and UN13 (when four differentials are considered) and 37 virulence combinations were categorized on 17 differentials. Virulence on the wheat differential carrying the *Lr26* gene was more common than in previous years. This gene is widely used by CIMMYT and other breeding programs, including those in Israel. No virulence was identified on differentials carrying the genes *Lr9* and *Lr24*.

Yellow rust – After years of sporadic occurrence, an outbreak of epidemic proportions took place in 1994. High incidence of this rust was recorded in the Hula Valley, the Bet She'an Valley and the Valley of Esdraelon, mainly in fields of the cultivar D'Ariel. The spread of the pathogen was halted by high temperatures in mid-March 1994. Eight cultures were sampled and studied on 13 USA differential varieties. A new virulence was recorded which was different from the five races identified in previous years by Dr. Z. Amitai. All eight cultures were virulent on cv. D'Ariel, which had been resistant to yellow rust in previous years. The new cultures were virulent on Heins VII, Veery "S" and TcLr26; the last two carry the rye translocation 1B/1R.

Stem rust – Two out of three cultures secured in 1994 were highly virulent on most of the Israeli cultivars and advanced breeding lines, including D'Ariel. Cv. Atir and genes *Sr13*, *Sr22*, *Sr32* and *Sr36* possess resistance to both cultures. (*L*)

Does Leaf Rust of Wild Relatives of Wheat Endanger Fields of Cultivated Wheat?

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Two different and distinguishable groups were found in studies conducted to clarify the biology of leaf rusts that attack wheat and its relatives. One group includes leaf rusts with alternate hosts of the Ranunculaceae family. The other group includes leaf rusts with alternate hosts of the Boraginaceae family. To elucidate the question of whether rusts from wild relatives of wheat will damage wheat, a broad range of wheat and wheat relatives' genomes, of various ploidy levels (2n, 4n, 6n), was inoculated with the various leaf rust types.

The results were as follows: *Triticum aestivum – Thalictrum* type attacks many species of *Triticum* and *Aegilops*. The *Triticum durum – Thalictrum* type also attacks species of *Triticum* and *Aegilops*, but less than does the *T. aestivum* type. The *Secale – Lycopsis* type attacks rye only. The *Aegilops longissima – Anchusa* type attacks mainly most of the species of the genus *Aegilops* that include the 'S' genome. The *Aegilops ovata – Echium* spp. type attacks mainly the 'UM' genome. The *T. durum – Anchusa italica* type attacks mainly *T. durum*. In view of the high level of specification, there does not seem to be any danger of transfer of leaf rust from wild relatives to cultivated wheat.

Breeders are using genes from wild relatives resources. Study of amphiploids, addition-lines and substitution-lines of wheat with *Aegilops* chromosomes has shown that when genomes or parts

of *Aegilops* or *Secale* genomes are added to wheat, the reaction to rust types from *Aegilops* or *Secale* is the same as to that from pure wheat. It seems that hexaploid wheat is generally resistant to wheat relatives-related rust types. This resistance is determined by the whole wheat genome and not by single genes. (*L*).

The Sexual Stage of the Common Maize Rust (*Puccinia sorghi*) – A Potential Threat to Corn Crops in Israel

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Common maize rust caused by the fungus *Puccinia sorghi* Schw. has a worldwide distribution. Generally, the damage to corn crops is insignificant, although there are reports of severe epidemics. The life cycle of the fungus is heteroecious. The pycnial and aecial stages occur on several *Oxalis* species. There are annual reports of occurrence of this fungus in cultivated corn fields in Israel. Disease severity is low in 'silage' cultivars. Sweet-corn cultivars are more susceptible and in some cases the disease severity is quite high.

Teliospores secured from sweet-corn leaves grown in a cultivated field in the northern part of Israel were artificially induced to germinate and produce basidiospores. The common garden weed *Oxalis corniculata* was inoculated under controlled conditions in the greenhouse with these basidiospores, and pycnia and aecia were formed on the leaves. We succeeded in inoculating corn seedlings with aeciospores from this alternate host. When corn straw bearing telia was placed next to *Oxalis* plants grown in a pot in a screenhouse at Tel-Aviv University, pycnial and aecial clusters were formed on the *Oxalis* leaves.

Our conclusion is that, although at present this disease is not of immediate danger to corn growers, the possiblity of the entire fungal cycle occurring in Israel may change this situation. As a result of the sexual stage, new combinations, some with a higher degree of virulence, may be formed. (*L*)

Why is the Rate of Rust Development in Sunflower Constant in Israel?

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Puccinia helianthi, the causal agent of sunflower rust, is widespread in most sunflower-growing regions in Israel. When disease appears early in the season, yield losses may exceed 90%. Analysis of disease progress curves recorded in three growing seasons (1991, 1992 and 1993) and three regions in Israel (Northern Negev, Lakhish and Hadera) revealed that the rate of disease progress was relatively uniform. The average apparent infection rate (r) was 0.237, which is a very rapid rate of disease development (*i.e.*, an increase in disease severity from 5% to 95% within 3 weeks).

Effects of microclimate parameters on components of the *P. helianthi* life cycle were studied under controlled environment conditions. Effects of temperature on spore germination were examined *in vitro*. The minimum, optimum and maximum temperatures for spore germination were 4, 4–15, and 27°C, respectively. At the optimum temperature range, the process was completed within 4–6 h. The influence of the duration of leaf wetness and temperature on the

infection process was studied *in vivo*. Within the range of optimum temperatures for spore germination, 6–10 h of leaf wetness were sufficient for completion of the infection process. Next, effects of temperature on establishment and sporulation were studied. The minimum, optimum and maximum temperatures for establishment were 5, 10–20 and 30°C, and for sporulation, 10, 15–30 and 37°C, in the same order.

Sunflower is grown in Israel in the summer. Disease onset usually occurs during May and the disease intensifies in June and July. During that period, conditions for spore germination and infection are optimal every night, and for establishment and sporulation – every night and day. Thus, disease development is not limited by the microclimate. These findings provide an explanation for the unusually uniform and rapid rate of rust increase in sunflower in Israel. (*L*)

Suppression of Pycnidial Protection on Wheat Seedlings Following Challenge Inoculation with Isolates of *Septoria tritici*

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The pathogen *Septoria tritici*, the causal agent of septoria tritici blotch of wheat, can cause severe yield losses in epidemic years. Resistance to the pathogen in wheat is expressed in low coverage by necrotic lesions and pycindia. Isolates of the pathogen infect wheat cultivars differentially.

Although on cv. 'Shafir' both Israeli isolates ISR398 and ISR8036 are virulent, pycnidial production on cv. 'Seri 82' following inoculation with isolate ISR398 is very low. The level of pycnidial coverage following inoculation of Seri 82 with a 1:1 mixture of conidia of the two isolates was significantly lower than the level recorded for isolate ISR8036 when inoculated singly, or from the mean of the two isolates on Seri 82. Challenge inoculations of Seri 82 with isolate ISR8036 – 2, 5, 6 and 10 days after inoculation with isolate ISR398, resulted in significant suppression of pycnidial production compared with seedlings inoculated with ISR8036 alone. No suppression was recorded on plants sprayed with the supernatant of isolate ISR398 and thereafter challenged by isolate ISR8036. Sub-isolates originated from pycnidia developed on Seri 82 following inoculation with isolate ISR398 and challenge-inoculated 5 days later by isolate ISR8036, were identified as ISR8036 by virulence test, on seedlings of Seri 82 and by probing fungal DNA with the minisatellite DNA probe ST398-3.7A. Sub-isolates which originated from pycnidia developed on seedlings of Shafir that were challenge-inoculated by the combinations ISR398(I)/ISR8036(II) and ISR8036(I)/ISR398(II), resembled in identity the isolate that was inoculated first in each of the two combinations. Demonstration of the presence of mycelium of each isolate in the leaf tissue following challenge inoculation at various time intervals prior to pycnidia production was performed using the ST398-3.7A probe.

The suppression of pycnidial production on Seri 82 with ISR398 and after that challenge-inoculated by isolate ISR8036 can be explained by cross protection, whereas the suppression recorded on Seri 82 and Shafir following inoculation with the reciprocal combination [ISR8036(I)/ISR398(II)] is explained by differential mycelial growth rate within the host tissue and competition between the two isolates within wheat leaf tissue. (*L*)

Sink-Source Relationships in Plants of the Wheat Cultivars Barkai and Miriam Infected with *Septoria tritici*

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Septoria tritici blotch of wheat (STB) incited by *Septoria tritici* Roberge ex Desmaz. causes severe losses in yield and in grain quality during severe epidemics. The broad virulence spectrum of the pathogen hampers the selection of resistant germplasm and the incorporation of resistance into commercial cultivars. The present resistance sources provide protection only against certain isolates but not against others, which dictates a breeding strategy adapted to a known virulence spectrum and which will still provide lasting protection. Genetic protection of yield in susceptible cultivars capable of sustaining yield (tolerance), can serve as an alternative to or complement resistance (tissue protection).

Losses of 15.8% in grain weight in cv. 'Miriam' as compared with 37.5% in cv. 'Barkai' were recorded under severe and equivalent STB epidemics. Degraining by half and mechanical removal of the flag leaves resulted in high compensation in grain weight of Miriam but not in Barkai. Evaluation of the soluble and structural carbohydrates in infected *vs* protected plants in different plant organs at different growth stages, provided no indication of reservoirs to be utilized later during grain filling, or conversion of structural carbohydrates to soluble, translocatable sugars. In addition, there was no translocation of soluble carbohydrates from lateral tillers to the central tiller. Fixation of CO_2 in healthy plants was similar in the two cultivars, but significant differences were recorded in *S. tritici*-infected plants. The CO_2 fixation in residual green leaf area in infected leaves of Miriam was at least twice as high as in protected plants, and in infected leaves of Barkai. It is proposed that the lesser vulnerability of Miriam to STB can be explained by an efficient fixation of CO_2 and production of assimilates in the residual green patches in infected plant organs, which thus serve as a compensatory mechanism for the loss in green leaf area. (*L*)

Epidemiology and Control of *Alternaria brassicicola* on Broccoli and Cauliflower

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Alternaria black spot caused by *Alternaria brassicicola* has caused severe yield and quality losses of broccoli and cauliflower during the past 5 years. Damage is expressed on both leaves and flowerheads. The disease develops mostly in autumn and early winter when temperatures are between 15 and 25°C and relative humidity >95%. The fungus secretes toxic substances which, at high concentrations, cause necrosis and wilting of seedlings of broccoli and all other brassica species tested; this phenomenon was never observed in commercial fields. Field experiments show that the disease can be controlled by Rovral (iprodione), Manzidan (mancozeb), Manebgan (maneb), Polyram (metiram), Score (difenoconazole), Mirage F (prochloraz zinc complex) or Impact (flutriafol), when they are sprayed before symptoms appear. Treatment with these fungicides in the field could also reduce postharvest damage effectively. Isolates resistant against

Rovral can be induced easily under laboratory conditions. Some resistant isolates showed high parasitic fitness on broccoli leaves, sufficient to compete with the sensitive wild type isolates. This fact should be considered in the control management of the disease. A high genetic variability exists in the population of *A. brassicicola* in Israel, which is expressed by: (i) aggressiveness variability; and (ii) tissue preference. Most isolates were able to infect all above-ground plant parts (leaf, stem and flowerhead). The flowerhead was found to be the most susceptible tissue. In conclusion, our results showed that: (*a*) when weather conditions are favorable for disease development, prophylactic treatments should be administered; and (*b*) the risk of development of resistant subpopulations of the fungus must be considered. (*P*)

E: PHYSIOLOGY AND GENETICS

Suppression of Colonization of Alfalfa Roots by a VA Mycorrhizal Fungus at High Phosphate Levels: The Involvement of the Plant Defense Response

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Vesicular arbuscular (VA) mycorrhizal fungi are obligate symbionts that form mycorrhizae with plant (*e.g.* alfalfa) roots. Although pathogenic biotrophs generally exhibit a high degree of host specificity, VA fungi show little to none. Indeed, mycorrhizae occur in approximately 80% of all plants. The major contribution of the VA fungi to the plant is improved phosphate (P) utilization. Furthermore, when P levels in the soil are optimal for plant growth, colonization of the plants by the fungi is suppressed. In this study, isoflavonoids and steady-state mRNA levels of phenylalanine ammonia-lyase (PAL), chalcone isomerase (CHI), and isoflavone reductase (IFR) were followed during a rapid, nearly synchronous infection by the VA fungus *Glomus intraradix* of alfalfa (*Medicago sativa* L.) roots treated with high or low levels of P, to test whether the host defense response is involved in the suppression of colonization observed at high P levels. In roots treated with the low P level, relative amounts of steady-state PAL and CHI mRNA increased between day 14 and 18, and then dropped rapidly to the control level (CHI) and even below (PAL). IFR mRNA was not induced by mycorrhizal colonization of the roots treated with the low P level. In these roots, HPLC (high performance liquid chromatography), proton-NMR (nuclear magnetic resonance), and FAB-MS (fast atom bombardment–mass spectrometry) analyses showed consistent increases in formononetin levels and transient increases in medicarpin-3-*O*-glycoside and formononetin conjugates when colonization began. As colonization increased, levels of formononetin conjugates declined below those in uncolonized controls. Medicarpin aglycone, an alfalfa phytoalexin normally associated with pathogenic infections, was not detected at any stage. Fluorescence microscopy did not reveal any lignification of the colonized tissue. When roots treated with the high P level were inoculated with *G. intraradix*, steady-state mRNA levels of PAL, CHI, and IFR were induced between days 15 and 20 and stayed above those of the control roots until colonization was finally successful, sometime between days 29 and 35. Furthermore, there was a consistent increase in medicarpin-3-*O*-glycoside and fluorescence microscopy hinted at lignification of tissue where penetration by the fungus had been attempted. We conclude that during successful colonization a limited plant defense response is induced, which is rapidly

suppressed. When colonization is suppressed by high P levels, a full defense response is detected. We postulate that this response is involved in plant regulation of colonization by the VA mycorrhizal fungus. (*L*)

Isolation and Identification of the Mycorrhizal Fungus *Tuber melanosporum* from Artificially Inoculated Oak Roots

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Fruit bodies of the black truffle of Perigord, *Tuber melanosporum*, imported from France and Italy, were used for inoculation of different oak species. The oaks, including local species that were unknown as hosts of this fungus, developed mycorrhizal complexes with typical mantle and 'puzzle' cells. To further verify the microscopic observations, attempts were made to isolate the fungus using various published methods. After several preliminary tests, modified MMN medium was chosen as the isolation and growth medium. Hydrogen peroxide or sodium hypochlorite at different concentrations and exposure times were tested to disinfect the mycorrhized roots. However, no *T. melanosporum* cultures were obtained. Therefore, a modified procedure for isolation of *T. melanosporum* from mycorrhized roots was developed. With this procedure high isolation percentages (up to 54%) were obtained. Isolates from five oak species: *Quercus boissieri* Reut., *Q. calliprinus* Webb, *Q. ithaburensis* Decne. (local species in Israel), *Q. ilex* L., *Q. pubescens* Willd. and from *Corylus avellana* L. (hazelnut) were compared with a reference culture obtained from G. Chevalier (INRA, Clermont Ferrand, France). Mycelium morphology and growth rate were identical. Genomic DNA was extracted from 11 isolates, from segments of four different truffles (from Italy) and from the reference French culture. PCR-amplification of the extracted genomic DNA, with three different primers, revealed identical banding patterns. (*L*)

Vegetative Compatibility among Isolates of *Colletotrichum gloeosporioides* from Almond in Israel

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In Israel, almond (*Prunus amygdalinum*) is grown mainly in two regions, the Yizre'el Valley and Lower Galilee in the north, and the Judean foothills and coastal plain in the south. Anthracnose, incited by *Colletotrichum gloeosporioides*, is the major disease of almond fruits in Israel. The disease was first recorded in 1977 in a few orchards in both the northern and the southern regions and reached an epidemic level in subsequent years. Pathogen attack of young fruits results in fruit rot and desiccation as well as leaf-wilting of distal clusters and, in extreme cases, shoot dieback. Seventy isolates of the pathogen were obtained from diseased fruits, collected during 1991/92 and 1994 from 11 sites in the two almond-growing regions. The isolates were uniform in morphology and mycelial growth rate (2.2 mm per day at 20–22°C).

Nitrate nonutilizing (*nit*) mutants were generated from each isolate and partially phenotyped as *nit 1* (52%), *nit 3* (24%), or Nit M⁻ (24%) using minimal medium supplemented with nitrate, nitrite or hypoxanthine. Mutants from different isolates were used to determine genetic relatedness among the isolates. Complementation (heterokaryon) tests showed that all 70 isolates belonged to a single

vegetative compatibility group (VCG). Tester mutants representing this VCG were not compatible with complementary *nit* mutants of *C. gloeosporioides* from anemone and avocado, indicating that the various pathotypes constitute distinct genetic populations. (*P*)

Vegetative Compatibility Groups of *Verticillium dahliae* in Israel

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189 isolates of *Verticillium dahliae* recovered from five host-plants at 22 sites in Israel were tested for vegetative compatibility using nitrate-nonutilizing (*nit*) mutants. Spontaneous *nit* mutants were generated from wild-type colonies by selecting chlorate-resistant sectors on cornmeal agar with glucose amended with potassium chlorate. Approximately 1200 *nit* mutants were recovered and divided into two phenotypic classes (*nit 1* and Nit M) based on their growth on nitrate minimal medium supplemented with nitrite or hypoxanthine. The majority (91%) were *nit 1* mutants. Complementation (heterokaryon) tests between *nit* mutants of different isolates led to identification of three distinct vegetative compatibility groups (VCGs). Distribution of isolates among the VCGs was: six in VCG I, 69 in VCG II and 114 in VCG III (designation of local VCGs, not international). Each of the main VCGs (VCG II and VCG III) seems to have specific regional distribution: VCG II, which includes isolates from eggplant, chrysanthemum and cotton, was distributed through the northern and central regions of Israel, whereas practically all the southern isolates from eggplant, cotton and potato were assigned to VCG III. Morphological differences were evident between isolates from different VCGs. This is the first indication of genetic heterogeneity in the *V. dahliae* population in Israel. (*P*)

The Use of Transmission Electron Microscopy and Pulse Field Gel Electrophoresis to Resolve Karyotypes among Plant Pathogenic Fungi

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Determinations of fungal karyotypes with light microscopy have been hindered due to the presence of numerous small chromosomes lying at the limits of resolution. Recently, reliable fungal karyotypes have been derived from computer-assisted, three-dimensional reconstructions of serially-sectioned meiotic pachytene nuclei analyzed at the ultrastructural level. A critical factor in these studies is the need to select precisely fusion nuclei in pachynema before synaptonemal complexes break up into nonresolvable elements. We have developed a technique whereby fusion nuclei are stained with DNA-binding fluorochromes, such that the same nucleus selected under epifluorescence microscopy can be processed for serial sectioning and electron microscopy. This technique has provided for the first reported karyotypes among the heterobasidiomycetes, including the important plant pathogens *Puccinia graminis* f.sp. *tritici* and *Melampsora lini*, in both of which n = 18.

Ultrastructurally derived karyotypes have recently been superseded by pulse field gel electrophoretic separation of intact fungal chromosomes. However, to date, such studies have included only a small number of representational isolates. We have extended the scope of analysis to compare statistically the limits of karyotypic variability among 118 isolates of the banana wilt pathogen *Fusarium oxysporum* f.sp. *cubense*, representing 15 vegetative compatibility groups (VCGs). The aim was to test whether variation in electrophoretic karyotype (EK) was correlated with presumptive clonal lineages as defined by VCGs in an asexual plant pathogen. Extensive EK differences were observed among the isolates: chromosome numbers (CN) ranged from nine to 14 (median 11, mode 12) and genome size (GS) ranged from 32.1 to 58.9 Mbp (mean ±S.D. = 43.3±5.8 Mbp). EK mean variation among 11 analyzed VCGs, however, was highly associated with VCGs, as determined by analysis of variance ($P<0.0001$). Comparison of means from both CN and transformed GS data sets identified two groupings of EK types containing identical VCG constituencies, which correspond to preexisting groupings based on host ploidy levels. (*P*)

Isolation and Characterization of Aflatoxigenic *Aspergillus* Strains Producing Aflatoxin and *Fusarium moniliforme* Producing Fumonisin B_1 from Sweet Corn Kernels in Israel

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Mycotoxins are highly toxic fungal metabolites produced by species attacking agricultural produce already at the preharvest stage. Corn kernels are attacked, *inter alia*, by *Aspergillus* and *Fusarium* species capable of producing mycotoxins in the field. Of the known mycotoxins, those belonging to the aflatoxins (produced by members of the *A. flavus* group), the trichothecenes and the fumonisins (produced by *Fusarium* spp.) are the ones most commonly found in corn kernels. The aim of this work was to isolate and identify the fungal strains of different corn varieties grown in Israel, to assess the ability of the strains to produce aflatoxins and fumonisin B_1, and to analyze the grains for the presence of these mycotoxins. This was done in an attempt to identify varieties resistant to fungal invasion and/or mycotoxin production. The varieties used in our study were 490, 887, 717 and 'Jubilee', all grown at Newe Ya'ar. Fungi were isolated from the grains using the direct plating and the dilution methods, and identified by the Centraalbureau voor Schimmelcultures (CBS), Baarn, The Netherlands. *A. flavus* and *A. parasiticus* (potential aflatoxins producers) and *F. moniliforme* (a potential fumonisins producer) were identified and used for further studies. Each of the strains was inoculated onto synthetic media (SM) and moist corn grain. In addition, SM and grains were inoculated with isolates *A. parasiticus* NRRL 5682 and *F. moniliforme* NRRL 13616, known to produce aflatoxins and fumonisins, respectively. All the *Aspergillus* strains tested produced aflatoxins both in SM and the corn grains. However, the two *Fusarium* strains produced fumonisin B_1 only on the grains, and not on SM. Neither aflatoxins nor fumonisin B_1 were found in any of the corn varieties tested. The ability of the fungi isolated here to produce mycotoxins in laboratory trials was shown. These strains are now being employed in studies aimed at the identification of corn varieties resistant to fungal invasion and/or for mycotoxin accumulation. (*L*)

Opening Lecture (by title only)

Molecular Approaches to Research on Plant Diseases

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Utilizing Antibodies and PCR for the Specific Detection and Estimation of Low Inoculum Levels of Aflatoxigenic Strains in Infested Grains

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Aflatoxins are highly carcinogenic metabolites produced by several species of fungi belonging to the *Aspergillus flavus* group. These species are common inhabitants of stored grain and their early detection is therefore of utmost importance for quality control management. Aflatoxins are synthesized *via* a unique pathway found in *A. parasiticus*. In the present study three genes coding for key enzymes have been identified: *apa-2, ver-1* and *omt-1*. Three primer pairs were generated, each complementing the 5' and 3' regions of the coding portion of one of the genes. The primer pairs were tested in the polymerase chain reaction (PCR) against DNA extracted from cultures of five *Aspergillus*, four *Penicillium*, three *Fusarium* and three insect species commonly found in stored grain and from peanuts and corn grains. Positive results (PCR product) were obtained exclusively with the aflatoxigenic molds *A. parasiticus* and *A. flavus*. The sensitivity of the method was studied by inoculating 1 g of sterile corn meal with spore suspensions of the different fungi at concentrations as low as 100 spores/g and incubating the samples in an enrichment medium. Aliquots of total DNA were taken periodically for PCR from the inoculated or sterile samples. Positive results were obtained after 24 h of enrichment only for corn inoculated with the aflatoxigenic molds. No PCR product was observed for corn inoculated with other fungi, even at the highest concentration of 10^6 spores/g after 72 h of incubation. Enrichment of grain samples followed by PCR was proven to be a more rapid and sensitive method than those in use today.

The amplified *ver-1* PCR product was cloned into an *Escherichia coli* expression vector (pGEX-2T) and the overexpressed chimeric protein was used to raise polyclonal antibodies in rabbits. Antibodies raised against the chimeric protein reacted specifically against mycelium extracts of *A. parasiticus* and *A. flavus*. Methods for improving the sensitivity of the ELISA reaction, by enriching antibodies using affinity chromatography, antigen fractionation, or various ELISA methods (ACP [antigen-coated plate], DAS [double antibody sandwich]), are currently being tested.

The integration of serological and molecular techniques should enable the rapid and accurate detection of aflatoxigenic fungi in grains and food products. (*L*)

Population Diversity of *Colletotrichum gloeosporioides* from Avocado and Almond Using Molecular Techniques and Pathogenicity Assays

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Isolates of *Colletotrichum gloeosporioides* from avocado and almond fruits were compared in order to determine the genetic diversity between and among the different populations. Four almond isolates exhibited very similar nuclear banding patterns compared with avocado isolates which had differential patterns. Similar results were observed with A+T-rich DNA representative of the mitochondrial genome. PCR amplification of genomic DNA using four primers grouped nine almond isolates from different geographic locations as uniform. In contrast, the avocado isolates were more diverse, with seven to ten different genotypes being observed. Amplification and subsequent restriction enzyme digestion of the 4–5 ITS region of ribosomal DNA failed to distinguish among isolates of *C. gloeosporioides* from a diverse host range. Avocado isolates produced varying lesions on avocado and almond fruits, whereas the almond isolates infected at a uniform rate. Certain avocado isolates seemed to produce perithecia in culture, whereas almond isolates remained asexual. This suggests that in asexually reproducing populations, such as the *C. gloeosporioides* almond isolates, little DNA variation is expected to occur in comparison with the sexually reproducing avocado isolates, where multiple genotypes are found. (*L*)

Use of RAPD Markers for the Identification of *Sphaerotheca fuliginea* Races

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Random amplified polymorphic DNA (RAPD) analysis has been applied for the identification of different isolates of *Sphaerotheca fuliginea*, the causal agent of powdery mildew in cucurbits. Isolates of the fungus were collected from various locations and hosts in Israel. Each isolate was grown on cucumber seedlings in separate growth chambers which contained additional melon plants (PMR 45 and PMR 6) as differentials for race identification. The isolates were maintained for further use by routine transfer on cucumber cotyledons placed on water agar medium amended with benzimidazole. Conidia were collected from heavily infested cucumber leaves. DNA was isolated by a micro-isolation procedure and subsequently subjected to RAPD analysis using various 10-mer primers. Ten *S. fuliginea* isolates (identified by differential plants) were tested with 209 arbitrary primers; 49 primers demonstrated polymorphism between isolates. Amplification with one primer produced a product that was amplified only in race 2 isolates. This primer was cloned for sequence-specific primer preparation. An additional strain that was isolated from watermelon was also identified; this isolate infected all differential plants, including PMR 45 and PMR 6, and appears unique in RAPD analyses, suggesting that this race may be new and undescribed in Israel. (*L*)

The Role of Plasmid-Borne Genes in Pathogenicity of *Erwinia herbicola* pv. *gypsophilae*

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Erwinia herbicola pv. *gypsophilae* (*Ehg*) induces gall formation in gypsophila. Our previous studies have shown that pathogenicity of *Ehg* is associated with a plasmid (pPATH) of about 150 kb. This plasmid contained genes for IAA (indoleacetic acid) biosynthesis (*i.e.*, *iaa*M and *iaa*H). Insertional inactivation of these genes caused substantial reduction in gall size. Further studies revealed a locus conferring cytokinin production which resided in a cluster with the IAA biosynthetic genes. Sequence analysis of this locus indicated the presence of a cytokinin biosynthetic gene (*etz*) homologous to the *ipt* and related genes. A unique open-reading frame (ORF) (pre-*etz*) of 169 amino acids preceded the *etz*. Northern analysis indicated the presence of an *etz*-specific transcript of 1 kb and a common transcript for the pre-*etz* and *etz* of 1.4 kb. A marker exchange mutant of the *etz* which was deficient in cytokinin production, exhibited a reduction in gall size on gypsophila cuttings. Insertional mutation in the pre-*etz* resulted in a sharp decrease in both the level of the *etz*-specific transcript and cytokinin production. Marker exchange mutation in the pre-*etz* exhibited reduced symptoms but to a lesser degree than the *etz* mutation.

In further studies the transposon-reporter Tn3-Spice was used to generate non-pathogenic mutants. Two clusters of mutants were obtained on the pPATH cosmid clone pLA150 containing the phytohormone genes. Most of these mutants have been restored for pathogenicity by complementation. DNA sequencing of two ORFs in this region showed a significant homology to genes involved in the protein secretion system (type III) which appears to function in pathogenicity of animal pathogenic bacteria. (*L*)

Assessment of Genetic Variation in *Septoria tritici* by Using a Minisatellite Probe Secured from the Pathogen

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The pathogen *Septoria tritici* Roberge ex Desmaz., the causal agent of septoria tritici blotch of wheat (STB), expressed a wide virulence spectrum and scarcity in resistance among commercial wheat cultivars. The categorization of virulence is based on the quantification of symptoms (necrosis and pycnidia) on differential wheat genotypes. The quantitative evaluation of host response introduces imprecision in assessing physiologic specialization in this economically important pathogen. The use of DNA probes enables assessment of genetic variation of different isolates of this pathogen, regardless of virulence. A wide genetic variation was revealed among *S. tritici* isolates by Southern analysis using the DNA-minisatellite probe 33.6, which originated from the myglobin gene of humans. This probe exhibited unique fingerprinting patterns for different *S. tritici* isolates. The fungal homolog of the minisatellite 33.6, referred to as 398–3.7A, isolated from chromosomal DNA of *S. tritici*, was cloned and sequenced. This sequence identifies a mean fingerprinting pattern of 12 bands per DNA of *S. tritici* isolate cut with PstI. The band pattern remains unique to each isolate and thus enables us to distinguish between the tested isolates. In addition, the probe 398-3.7A revealed differentiated DNA fingerprints of the wheat pathogens

Bipolaris sorokiniama and *Stagnospora nodorum*. The core of the 398-3.7A sequence is built from 23 repeats rich in guanine, and is composed of three elements: $(GGN)_2$; a consensus sequence of 12 bases (GGAGGACAGGGC); and a sequence of 18 bases which appear in two distinct forms. The 12-nucleotide consensus sequence shows high homology (>90%) to the core sequence of the human 33.6 minisatellite and to other closely related minisatellites, despite the evolutionary distance and the lack of linkage to a defined function.

The minisatellite probe 398-3.7A from *S. tritici* will enable elucidation of biological, genetic and epidemiological aspects associated with this pathogen. (*L*)

Conidiophore Development in *Neurospora* is Regulated by *rco-3*, a Gene that Shares Homology with Hexose Transporter

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Neurospora crassa produces three types of spores: two asexual spores, macroconidia and microconidia, and sexually derived ascospores. Conidia formation is influenced by nutritional status; limitation of carbon or nitrogen can induce conidiation in submerged culture, whereas with adequate carbon and nitrogen sources conidiation occurs only at an air interface, and is also influenced by light. Internal cues, such as the circadian rhythm, can affect the timing of development. Genes that are expressed during conidia formation are also subject to complex regulation. The *con-10* gene is expressed during macroconidiation and regulated in response to light and to circadian rhythm, and during formation of the other types of spores. We have selected mutants that express *con-10* under conditions of constant light and submerged growth. One such mutant was able to undergo conidiation in submerged culture with sufficient nitrogen or carbon sources. The gene responsible for this phenotype, *rco-3*, was cloned. The sequence analyses of *rco-3* revealed homology to the sugar transporter gene superfamily, with greatest identity to *Saccharomyces cerevisiae snf-3*, a gene encoding for the high affinity glucose transporter. Inactivation of *rco-3* in the wild type strain by subjecting it to Repeat Induced Point Mutation, or by gene replacement, yielded progeny with a phenotype similar to the *rco-3* mutant. The growth properties of the mutant suggest that this gene may be generally involved in regulation of carbon source transport and metabolism. (*L*)

Inactivation of a Single Type 2A Protein Phosphatase is Lethal in *Neurospora crassa*

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Serine/threonine-specific protein phosphatases have been identified and classified into four major subgroups (PP1, PP2A, PP2B and PP2C) according to their substrate specificity, dependence on divalent cations and sensitivity to inhibitors. In each of the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, at least two genes encoding PP2A catalytic subunits have been identified, neither of which is essential.

A polymerase chain reaction approach, employing degenerate oligonucleotide mixtures, was used to isolate *pph-1*, a type 2A protein phosphatase (catalytic subunit)-encoding gene, from

Neurospora crassa. The isolated single-copy gene is 1327 nucleotides in length, contains four putative introns and encodes a 310 amino acid polypeptide. *pph-1* is located between *pdx-1* and *col-4* on the right arm of *N. crassa* linkage group IV. *pph-1* transcript levels are highest during the first hours of conidial germination. Failure to obtain viable progeny in which *pph-1* had been inactivated *via* the Repeat-Induced Point mutations process and evidence that nuclei harboring a disrupted *pph-1* gene could be maintained only in a heterokaryon, indicate that a functional *pph-1* gene is essential for fungal growth. This is the first report providing evidence that inactivation of a single type 2A protein phosphatase results in a lethal phenotype in fungi. (*L*)

Regulation of Sexual Reproduction in Pathogenic Fungi

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Many fungi of agricultural or industrial importance are not amenable to conventional genetic analysis because they cannot reproduce sexually. In sexually reproducing fungi, mating is controlled by master regulatory genes; these mating type (MAT) genes have been cloned from a number of fungi including the pathogenic Ascomycetes *Cochliobolus heterostrophus, C. carbonum* and *C. victoriae*. Surprisingly, *C. heterostrophus MAT2* homolog was found in *Bipolaris sacchari*, an asexual relative of *C. heterostrophus* (anamorph of *Cochliobolus* is *Bipolaris*). We have determined that the *MAT-2* homolog of *B. sacchari* is functional in *C. heterostrophus*. Moreover, introduction of *C. heterostrophus MAT* genes into *B. sacchari* allows the transgenic *B. sacchari* strain to initiate (but not complete) mating with a *C. heterostrophus* strain of opposite mating type, although fertility of such crosses is very low. Such strains do not cross with *B. sacchari* and without the transgene, the *B. sacchari* strain does not cross with *C. heterostrophus*. Northern analysis has indicated that the *B. sacchari MAT2* gene is not expressed in *B. sacchari* under conditions that normally induce *MAT* transcription in *C. heterostrophus*. However, *MAT* transcript is detected in transgenic strains of *B. sacchari* carrying several copies of *C. heterostrophus MAT* genes. These results suggest that *B. sacchari* lacks functions other than MAT which are essential for mating. (*L*)

G: USE AND APPLICATION OF NEW AND ESTABLISHED PESTICIDES

Frowncide – A New Fungicide for Treatment of a Wide Range of Diseases

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Frowncide (fluazinam; also called Shialan) is a fungicide/acaricide from Ishihara Sangyo Kaisha (ISK) Ltd., Tokyo, Japan. It acts by delaying the process of oxidative phosphorylation; this mode of action allows Frowncide to be effective against a wide range of pathogens (bacteria, fungi) and acarines (mites) in plants. The material is highly efficacious, especially in prophylactic

applications, withstands rain and irrigation, and has a long persistence. Frowncide is formulated as a 50% fluazinam SC.

Potatoes: Tests *in vitro* showed good activity in delaying the onset of late blight disease, *Phytophthora infestans*. In greenhouse experiments it prevented the growth of *Phytophthora* in strains susceptible as well as resistant to metalaxyl. In field experiments in different regions of Israel, better control was obtained with Frowncide by spraying 0.5–1 *l*/ha before the appearance of late blight than was obtained by treatments with Mancur (cymoxanil + mancozeb), propamocarb or mancozeb. At this application rate, good control was obtained also against early blight, *Alternaria solani*.

In tests *in vitro*, Frowncide was as effective as Benlate (benomyl), completely inhibiting the growth of white mold, *Sclerotinia sclerotiorum*, the causal agent of diseases of a wide range of crops.

Tomatoes for Processing: In experiments in greenhouses, Frowncide prevented the growth of *Phytophthora* in strains susceptible as well as resistant to metalaxyl. In field experiments Frowncide prevented the growth of *Phytophthora* at an application rate of 1 *l*/ha. It also controlled a rust mite, *Vasates lycopersici*, the carmine spider mite, *Tetranychus cinnabarinus*, and the twospotted spider mite, *Tetranychus urticae*.

Carrots: In field experiments Frowncide prevented the spread of leaf blight, *Alternaria dauci*. Treatment with Frowncide at a rate of 0.5–1 *l*/ha was more effective than mancozeb formulations.

Peanuts: In laboratory tests, rates as low as 1 ppm were effective in preventing the growth of isolates of scab-forming *Streptomyces* spp. originally isolated from peanuts. In field experiments in the Negev, preplant application of Frowncide reduced the occurrence of scab disease; in addition, there was a reduction in the occurrence of net blotch on the peanut shells and in the occurrence of *Maladera matrida* grubs in treated as compared with control plots.

Vineyards: In field experiments in different regions of Israel, concentrations of 0.1% Frowncide effectively prevented the occurrence of grey mold, *Botrytis cinerea*. Application of Frowncide at this rate was also comparable to application of 0.15% iprodione against *Botrytis*. Frowncide at 0.1% was comparable to 0.4% Sandocur–copper (copper oxychloride + cymoxanil + oxadixyl) against *Plasmopara viticola*. (*L*)

Nebijin (MTF-651), A New Product to Control Soilborne Diseases

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Nebijin is a new product of Mitsui Toatsu Chemicals Ltd., Tokyo, Japan, for the control of fungal and bacterial diseases in the soil. It is formulated as a 5% flusulfamid SC.

It was found in laboratory experiments that Nebijin is effective at a concentration range of 1–5 ppm against *Xanthomonas campestris* pv. *vesicatoria*, which causes black rot in tomatoes and fresh market peppers, and against *Streptomyces scabies* causing common scab in potatoes. Further, it was tested against *Streptomyces* spp. causing scab in peanuts and found to be effective at a concentration of 1 ppm.

The efficacy of this product in controlling soilborne bacteria was assayed by three application methods: (i) semi-field application against scab disease in peanuts; (ii) soil application against scab disease in peanuts; and (iii) foliar application against black rot in radishes.

Semi-field application: Pathogen-infested soil (*Streptomyces* spp.) was mixed with Nebijin at a concentration of 2 or 4 ppm a.i. and put in containers. Peanuts were planted in them and observed for incidence of scab disease. At these two cncentrations, disease level was reduced by 22% and 44%, respectively, compared with the level in untreated containers.

Pre-plant soil application: Nebijin was applied at a rate of 40 *l*/ha and disced into pathogen-infested (*Streptomyces* spp.) soil to a depth of 20 cm. Leaching was minimal as residues were found no more than 5 cm from the treated soil layer. Peanuts were planted and observed for scab disease. Treated plots had approximately 50% incidence of scab disease compared with the untreated plots.

Foliar application: Field-grown radishes infected with black rot (*X. campestris*) were treated with various concentrations of Nebijin. At a concentration of 4 *l*/ha there was 7% damage, while control plants had 22% damage. In field experiments in which timing of application was varied, it was found that there was no difference in effectivity between different application times.

In summary, the field experiments with Nebijin are just at the beginning, but the product shows promise as an effective and long-lasting compound for control of soilborne bacterial diseases. (*L*)

Control of *Phytophthora infestans* in Potatoes and *Pseudoperonospora cubensis* in Cucurbits by Dimethomorph + Mancozeb (Acrobat)

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Dimethomorph (DMM) is a systemic fungicide for controlling oomycete fungi such as *Phytophthora infestans* causing late blight in potatoes, and *Pseudoperonospora cubensis* which causes downy mildew in cucurbits. DMM is a cinnamic acid derivative with a unique mode of action. It is the first oomycete fungicide to disrupt cell wall formation. Whereas many other fungicides inhibit fungal development, DMM actually causes cell wall lysis, resulting in death of the fungal cell. DMM is active at all stages of the fungal life cycle, except zoospores release from zoosporangium, a stage which is highly sensitive to mancozeb (MZ).

Acrobat contains 90 g DMM and 600 g MZ per kg product. Foliar applications of DMM give good protectant, curative and excellent antisporulant activity and provide long residual protection. DMM is translocated systemically in the plant when applied to the roots, and is translaminar with local systemicity when applied to the foliage. No cross resistance to DMM was observed in phenylamide-resistant fungi.

A few field trials were carried out to control *P. infestans* late blight in potatoes during the year 1993/94. It was found that spraying Acrobat at 10-day intervals in potatoes var. 'Mondial' controlled the disease efficiently and raised the yield by 24% compared with propamocarb hydrochloride, 14% above metalaxyl MZ (Ridomil-MZ W.P., containing 7.5% metalaxyl and 56% MZ) and 15% above MZ. Mixing Acrobat with Ridomil-MZ at lower rates in tank-mix improved the control achieved by the mixture, more than both of them in single application. Spraying Acrobat at 10-day intervals controls efficiently downy mildew caused by *P. cubensis* in field and greenhouse. No advantage was gained by mixing Acrobat with Ridomil-MZ for downy mildew control. (*L*)

Dimethomorph's Activity against Oomycete Fungal Plant Pathogens

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Dimethomorph (DMM) was effective in controlling late blight in potato and tomato caused by either metalaxyl-sensitive (MS) or metalaxyl-resistant (MR) field isolates of *Phytophthora infestans*, and downy mildew in cucumbers and melons caused by MS or MR isolates of *Pseudoperonospora cubensis*. The fungicide did not affect zoospore discharge from sporangia of *P. infestans* but strongly inhibited zoospore encystment, cystospore germination and mycelial growth *in vitro*. DMM showed translaminar activity and local systemic activity in intact plants but failed to translocate from one leaf to another in either acropetal or basipetal direction. DMM applied as a soil drench was effective in protecting tomatoes against late blight but failed to protect cucumbers against downy mildew. When applied in lanolin paste to the stem surface, but not when applied to the hypocotyl, it provided excellent control of both diseases on foliage. DMM applied 1 day postinoculation protected potato against late blight and cucumbers against downy mildew only partially but in both systems it reduced sporulation of the pathogen. DMM diminished the sporulation of *P. infestans* and *P. cubensis*, when applied to normally developed infected tissue. In *P. cubensis* it induced enhanced callose-encasement of haustoria. The fungicide showed a remarkable persistence on foliage following excessive washing with water as well as a high residual activity lasting for 9 days. DMM seems to be a good candidate for the control of oomycete diseases in the field, especially in growing areas where phenylamide-resistant fungal populations prevail. *(P)*

Difenoconazole (Score) is Efficient for Control of the Leaf Withering Disease of Onion and Garlic Caused by *Stemphylium* sp.

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Onion and garlic (Liliaceae) are two economically important crops that are severely affected by the fungal pathogen *Stemphylium* sp. Garlic is infected during late winter and spring, whereas onion is infected in spring and summer. In both cases the infection results in leaf withering that shortens the length of the growing period of the crop. Leaf withering starts at the lower leaves and advances to the upper part of the plant. In both crops yield losses are estimated at 20-30%. The spread of the fungal spores is enhanced by rain in the winter, and by dew during the summer. Onion and garlic varieties differ in their susceptibility to the disease.

In field experiments carried out in 1991–93 we tested the systemic fungicide difenoconazole (Score, EC 250) as to its efficacy in controlling the disease.

Difenoconazole applied at 190 g a.i./ha (760 ml Score/ha) was efficient in controlling the disease and increased the yield of onions (var. 'Eitan') by 26%. Sprays were administered four times, at weekly intervals. In other experiments at Kibbutz Dorot, Kefar Bialik, Kefar Kara' and Kibbutz Elrom, yields increased by 17–31%. In these cases application was 125–250 g a.i./ha (0.5-1 *l* Score/ha). The increase in yield was correlated closely with higher application rates of the fungicide. With high application rates the weight of the plant canopy increased by 86% compared with the untreated control. Score was also efficient in the control of rust.

The use of the systemic fungicide Score can be an important element of the spraying regime against *Stemphylium* sp., in combination with the traditional protectant fungicides. (*L*)

The Influence of Spray Drop Size and Density on Control of *Botrytis cinerea* on Rose Petals

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Runoff sprays applied in rose greenhouses result in an uneven deposition of the fungicides and in a significant loss of pesticide to the soil. The continued public interest in decreased use of pesticides calls for research on improved application techniques such as reduced volume application. The latter is achieved with discrete drops which do not unite on the plant surface, are evenly deposited and the distribution of which is controlled in terms of uniformity and density. The present study focused on the relationships between fungicide deposit, droplet distribution and the prevention of rose petal infection caused by *Botrytis cinerea*.

The fungicides used throughout this work were Mythos FC (30% pyrimethanil), which is characterized by translaminar mobility and high vapor pressure, and the formulated mixture Mirage F WP (15% prochloraz zinc and 60% folpet), which is a protectant. Detached rose petals were inoculated by spray of a conidial suspension of the pathogen and left to dry. The petals were then subjected in an exposure chamber to sprays which were characterized by various droplet sizes (80–200 μ volume median diameter) and densities of 60–1000 droplets/cm^2. Various combinations of droplet size and density but resulting in an identical total deposit value were sprayed. The treated petals were incubated in humidity chambers. The potential of vapors of the fungicide to control Botrytis petal blight was tested as follows: rose petals were placed on wet filter paper in the bottom halves of petri dishes. The latter were then covered with the lids, to the inner sides of which the fungicides had been applied; the two halves were then sealed together with parafilm strips.

Botrytis petal blight was prevented by Mythos applications which resulted in residual deposition in excess of a certain threshold, regardless of the density of deposition. The disease was also controlled by the vapors of Mythos. Mirage F applied at a high density (1000 droplets/cm^2) controlled the disease more effectively (88% disease prevention) than an identical deposition achieved by lower droplet density (500/cm^2) resulting in only 40% disease prevention. Similar results were obtained in the past with the mixture of carbendazim and diethofencarb (WPs, 25% each). Vapors of Mirage F did not affect petal blight.

It may be concluded that the efficacy of protectant fungicides is improved by an increased droplet density, whereas droplet density is less important when the fungicide is characterized by efficient secondary distribution due either to high vapor pressure or to translocation ability in the host plant tissue. (*L*)

Rugby – A New Nematicide in Israel

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Rugby (cadusafos) (also called Apache or Taredan) is an organophosphorus nematicide–insecticide manufactured by FMC Corp., Philadelphia, PA, USA. The chemical has low solubility in water, limited movement in the soil and a prolonged breakdown period, properties which ensure long activity in the rhizosphere. As the chemical is not systemic, no danger exists of residues remaining within the edible parts of the crop.

Rugby is marketed as a 100 g a.i./*l* micro-emulsion (ME) in a water-miscible formulation. It is recommended to apply the product on the soil surface and then to incorporate it prior to seeding or planting. It can be applied also through the drip irrigation system in existing banana plantations.

During 1993 and 1994 efficacy trials were conducted in Israel to study its ability to control the root-knot nematode [RKN] (*Meloidogyne javanica*) in vegetables, and the spiral nematode (*Helicotylenchus multicinctus*) in bananas.

In an experiment conducted in a RKN-infested squash field, Rugby at rates of 20, 40 and 60 *l*/ha was compared with Nemacur (fenamiphos 40% a.i.) at 20 *l*/ha, and with an untreated control. One month after germination, the untreated roots were heavily galled and had a galling index of 4.7 on a 0–5 scale, whereas the roots in both the Rugby and the Nemacur treatments had a galling index of approximately 2.5. Two months after germination, the roots in the untreated plots had a galling index of 5; Nemacur-treated plots, 4.5; and the Rugby treatments, 2.5. Fresh plant weights in the plots treated with 20 and 40 *l* Rugby/ha were 50% higher than the Nemacur treatment and untreated plants; the latter were 1000 g/plant. The yield of the untreated control plot was 17.6 kg, and that of all the other treatments was 30–40% higher.

In a similar experiment conducted in a RKN-infested cucumber field, Rugby and Nemacur were applied as described above for squash. One month after germination, the average galling index of the roots in the untreated and the Nemacur-treated plots was 4, whereas for the Rugby treatments, the index was 3. Plant fresh weight of both the untreated and the Nemacur treatment was only 60 g, compared with 250 g/plant for the Rugby treatments. The average yield/plot in the untreated plots was 7.6 kg, 10.4 kg in the Nemacur plots and 18.0 kg in the Rugby plots at the 30 and 40 *l*/ha rates.

In a long-term field trial conducted in a banana plantation in the Jordan Valley, Rugby at 4 and 6 g a.i./mat and Nemacur at 9 g a.i./mat were applied twice a year, in June and in August. Evaluation of spiral nematode infection (on a scale of 0–5) in 1993, showed 2.1 for untreated, 2.06 for Nemacur, 1.96 for Rugby 4 g a.i., and 1.56 for Rugby 6 g a.i. In 1994, the Rugby infection index was 1.2, whereas all the other treatments had an infection index of approximately 2.0. Taking as criteria productive parameters such as flowering dates, fruit size and bunch weight, Rugby showed an advantage over the other treatments. (*L*)

Morphological and Cytological Changes Induced in Plant Roots by the Root-Knot Nematode *Meloidogyne artiellia*

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More than 50 species of *Meloidogyne* are known, four of which are of special agricultural importance and widespread in subtropical and tropical areas, including Israel: *M. incognita, M. arenaria, M. hapla* and *M. javanica.* On the other hand, the distribution of *M. artiellia* is restricted mostly to the Mediterranean region. In Israel this nematode is found in the northern Negev. The four species mentioned above have a very wide host range, whereas that of *M. artiellia* includes plants in only three families: Papilionaceae, Graminaceae and Cruciferae. Except for some knowledge about distribution and host range, no further information about this species is available.

Our results indicate that the morphological and cytological changes induced in roots by *M. artiellia* are different from those induced by the other *Meloidogyne* species mentioned above. In these other species the feeding site includes four or five nurse cells that undergo processes of hypertrophy and hyperplasia, that finally form multinucleate giant cells – coenocytes. Hypertrophy of cells in the parenchyma of the root cortex is observed. All these tissues together form the root gall. On the other hand, in *M. artiellia* the changes in host tissue following nematode parasitism (in roots of *Brassica* sp.) result in hypertrophy and hyperplasia of the nurse cells, but no multinucleate giant cells are formed. Instead, a syncytium (composed of the nurse cells after the cell wall collapses) is observed. Small galls are formed, which are difficult to notice under field conditions.

The formation of a syncytium and the amphimictic production of *M. artiellia* may indicate a different evolutionary development of this species compared with the other *Meloidogyne* species. (*L*)

Attachment of *Pasteuria penetrans* Spores to Second-Stage Juveniles of the Root-Knot Nematode *Meloidogyne javanica*

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The bacterium *Pasteuria penetrans* is a natural enemy of several nematode species, including the root-knot nematode *Meloidogyne javanica*, a very important pest with a multiple host range. The limiting factor in the application of this biocontrol agent is its obligate nature of parasitism on nematodes.

The bacterial spores attach to the second-stage juveniles (J2) in soil and, after root penetration, the bacterium completes its life cycle inside the nematode. The spores attach to the outermost layer of the nematode – the surface coat. This layer contains proteins, glycoproteins and carbohydrate recognition domains.

Our work indicates the involvement of a 250 kDa protein in the sporebinding process to the nematode. Polyclonal antibodies that recognize this antigen, labeled the nematode surface, except for the head region. Scanning and electron microscopy techniques were used to localize the sites of

antibody labeling. Binding of the antibodies to J2 significantly reduced the spore attachment to the nematode; however, this inhibition was restricted to the region of antibody binding. Pretreatments of the spores with surface coat extract inhibited the spore attachment as well, in the body region, but not at the head region of the juvenile.

Carbohydrate binding proteins present on the nematode surface may also be involved in spore attachment, because pretreatment of the J2 with fucose or mannose reduced the attachment. The 250 kDa fragment does not contain carbohydrate recognition domains.

An understanding of the attachment mechanism and characterization of the nematode surface may contribute in the future to the development of alternative strategies for nematode control. (*L*)

The Structure and Function of the Root-Knot Nematode's Gelatinous Matrix

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The gelatinous matrix (GM) of the root-knot nematode (*Meloidogyne* spp.) is produced by six rectal gland cells arranged radially around the female anal opening. The GM is secreted through the anus in voluminous amounts before and during the egg-laying period, and the nematode eggs are deposited into it to form the egg-mass. In a light microscope study of a *M. incognita* monoxenic culture on excised tomato roots, the development of the egg-masses was observed daily. Two phases were distinguished in the GM: a clear amorphous hyaline substance located at the periphery of the egg-mass, which is most probably secreted by the nematode first; and a fibrillar substance of yellowish-brown color tending to become darker with time, which comprises the bulk of the GM and into which eggs are deposited. Four-week-old *M. incognita* egg-masses were studied with a low-temperature scanning electron microscope. The two distinct phases were observed; no structural patterns could be distinguished in the hyaline substance at the egg-mass periphery. The fibrillar phase was found to have a delicate porous mesh structure. The pores among the fibrils were of various sizes: in fresh GM the pores were small, whereas in the older GM the pores occupied most of the GM volume. Tiny pearl-like structures were observed along the fibrils.

The GM was found to dissolve the gall tissues to form a canal leading from the nematode's posterior region to the gall surface. The lysis of the gall tissue suggested the presence of cellulytic enzymes in the hyaline phase of the GM. The survival of the egg-mass in the soil hinted at an antimicrobial protection mechanism. Indeed, it was found that the GM has such properties, binding microorganisms coming in direct contact with it. The fact that root-knot nematode eggs could survive in extremely dry soil suggested that the GM maintained a moist environment for the eggs, avoiding their desiccation. (*L*)

Involvement of RNA-1 of Cucumber Mosaic Virus in Viral Movement in Squash

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The differential rate of systemic symptom induction in zucchini squash by the Fny- and Sny-strains of cucumber mosaic virus (CMV) previously was mapped to RNA 1, which encodes a protein (1a) involved in virus replication. Examination of the kinetics of accumulation of the RNAs and four encoded proteins in the inoculated cotyledons and in the systemically infected leaves showed that Fny-CMV-associated products generally appeared earlier than the Sny-CMV-associated products. However, both Fny-CMV and Sny-CMV RNAs showed similar kinetics of RNA, 2a, 3a and coat protein accumulation in protoplasts prepared from zucchini squash cotyledons. These data indicate that the differential rate of systemic symptom development was due to a difference in the rate of movement rather than of replication. This was confirmed by a leaf-detachment assay, which showed a difference in the rate of systemic movement by Fny-CMV *vs* Sny-CMV; and by leaf-press blot hybridization of the inoculated cotyledons at different days postinoculation, which exhibited a difference in the rate of cell-to-cell movement by the two strains of CMV. Taken together, the data demonstrate that the rates of cell-to-cell and the long-distance movement can be regulated by sequences in CMV RNA 1, previously thought to be involved only in virus replication. (*L*)

Preparation of a Riboprobe for Detection of Pelargonium Flower Break Virus

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Pelargonium (geranium) is probably the largest selling ornamental crop in the world. Israel exports cuttings and rooted plantlets to Europe. The European and Mediterranean Plant Protection Organization (EPPO) has proposed guidelines which will serve as the basis of the EU plant protection regulations. Of the eight viruses included in these guidelines, the following three have been identified in pelargonium nurseries in Israel: pelargonium flower break virus (PFBV), pelargonium line pattern virus (PLPV) and pelargonium leaf curl virus (PLCV). Specific antisera to these three viruses were produced by us and are presently used in ELISA to test nuclear stock nurseries. In these tests a substantial number of PFBV-infected plants were detected and a small number of PLPV-infected plants. However, in repeat tests of PVBV-negative reacting plants, a small number of PFBV-positive reacting plants were detected even after two repeat tests. Since no mechanical or insect transmission of PFBV was evident, it seems that under certain conditions (high temperature?) PFBV concentration in the plants at the first testing was below the level detectable by ELISA.

Due to the fact that several reports showed riboprobes to be more sensitive than ELISA, we developed a riboprobe for PFBV. cDNA to the viral RNA was cloned into a plasmid and then into

a transcription plasmid. The radioactive labeled transcript (riboprobe) hybridized with viral RNA immobilized on a nylon membrane, and enabled detection of PFBV even at a dilution of 1:6000 of the plant extract. The sensitivity of the riboprobe compared with that of ELISA was discussed. (*L*)

Rapid Diagnosis of Grapevine Virus Diseases – *In Vitro* Methods

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Grapevine leafroll and corky bark are virus diseases, which reduce the quantity and quality of the yield and may cause serious economic damage. Grapevines are propagated vegetatively by cuttings or grafting. As the diseases are graft-transmissible, a rapid and reliable method for identification of viral diseases is important for establishing virus-free propagation stocks, introduction of new varieties, etc. Until now the diseases have been diagnosed by grafting cultivars onto indicator plants, requiring as long as 2–3 years to complete the diagnosis. Two tissue culture methods were developed in order to shorten the diagnosis time.

i) Micrografting. Infected explant shoots were micrografted onto healthy indicator plants, and maintained *in vitro*. Typical corky bark symptoms appeared on the indicator plant after 8–12 weeks. Affected plants were stunted, and displayed red, down-rolling leaves, and swelling and longitudinal cracks on the stem.

ii) Leafroll disease symptoms sometimes resemble stress symptoms. In tissue culture, osmotic stress induced by sorbitol enhanced disease symptoms. Distinct symptoms – inhibition in shoot and root development, down-rolling and reddening of leaves – were observed after 6–8 weeks.

The two methods described shortened the indexing time significantly, and indicated the presence of disease regardless of the virus associated with it. The methods are simple to apply and can be performed the year round in a growth chamber. They should be developed further so that they will be suitable for other virus diseases. (*L*)

Characterization of the Biology and the Epidemiology of Tomato Spotted Wilt Virus

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Tomato spotted wilt virus (TSWV) has been classified as the sole member of the newly created genus, *Tospovirus*, within the Bunyaviridae. The virus is transmitted exclusively in a persistent manner by nine thrips species; the Western flower thrips, *Frankliniella occidentalis* (Pergande), is the most efficient. TSWV was detected in Israel in 1991. The virus is well known for its broad host range, which includes more than 500 species occurring in over 50 families. It causes severe outbreaks in a considerable number of economically important ornamental and vegetable crops.

The aim of the research presented herein was to identify the natural host range of TSWV. Weed species and native plants were collected in the Sharon valley and the north of Israel. These included 128 samples of 16 plant species representing 13 families. Samples were assayed by the enzyme-linked immunoabsorbent assay (ELISA), using a commercial kit against the BR-01 isolate of TSWV, and by bioassay on suitable indicator plants. The virus was detected in six species

including *Sonchus oleraceus, Solanum niger, Portulaca oleracea, Erigeron crispus, Silybum marianum* and *Cichorium pumilum*. Primary and secondary spread of the virus in commercial fields of lettuce, tomato and pepper was studied at various seasons throughout the year. Colored sticky traps were used for monitoring thrips populations. Infection incidence ranged between 3% and 100%. The virus was detected mainly in species within the Compositae. A focal spread pattern was found in lettuce and tomato fields in Qiryat Bialik and Kibbutz Osha, respectively. Infection rates were low in lettuce (1–5%) and high in tomato (10–50%). A significant correlation between thrips numbers and TSWV disease incidence was recorded.

In thrips transmission studies, the virus was successfully transmitted from TSWV-infected *Datura stramonium*. Variable virus transmission efficiency to healthy *Datura* and pepper plants has been observed. Further studies have been initiated to achieve a better understanding of the epidemiology of this disease. Distribution of reservoir plant hosts for the virus, the efficiency of transmission to economically important crops, and the importance of other environmental factors in determining disease outbreaks are currently being assessed. (*L*)

Virus Diseases in Petunia

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The genus *Petunia* constitutes herbaceous ever-blooming ornamentals. In nature, they are propagated sexually by seeds. Recently, new species of vegetatively propagated petunia ('Supertunia') were introduced and they are gaining interest as a garden ornamental in Europe and the United States. A number of Israeli growers have started to export petunia cuttings to Europe. In 1993/94, about 3 million cuttings were exported.

Petunia is subject to several virus diseases. During surveys of petunia nurseries in Israel, three viruses were identified: cucumber mosaic virus (CMV), potato virus Y (PVY) and tomato yellow leaf curl virus (TYLCV). The first and second are transmitted in a nonpersistent manner by aphids and the third is vectored by *Bemisia tabaci*. Petunia has been reported as a host for tomato spotted wilt virus (TSWV) transmitted by thrips, alfalfa mosaic virus (AMV) transmitted mechanically and in a nonpersistent manner by aphids, and tobacco mosaic virus (TMV) transmitted mechanically and through seeds.

Virus diseases of petunia are a major threat to the production and quality of the crop. Propagation material from virus-infected mother plants will automatically transmit the virus from generation to generation. Therefore, suitable and sensitive virus indexing methods and certification procedures will have to be established to assure production of virus-free mother plants of petunia.

In order to maintain the horticultural characteristics of vegetatively propagated petunia cultivars, a meristem tissue culture propagation procedure was developed at the Dept. of Virology, ARO, in cooperation with the Danziger Farm. The procedure will be utilized for producing virus-free mother plants of petunia. (*L*)

Status of Lily Growing in Israel

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Lilies are grown in Israel for cut flowers and bulbs, the latter for both export and local use. The crop is often severely affected by various virus diseases, which cause mosaic and necrosis on the leaves, stunting of the plants and reduced quality of the flowers. The following viruses have been identified in lilies grown in Israel: cucumber mosaic virus (CMV), lily symptomless virus (LSV), lily mottle virus (LMoV) and strawberry latent ringspot virus (SLRV). The first three are transmitted by aphids in a nonpersistent manner; SLRV is transmitted by nematodes. Lily virus X (LVX) was detected in the laboratory using ELISA but this finding has still to be corroborated using an additional method of identification. Infection by viruses has led previously to a complete degeneration of lily stocks.

The success of lily production depends, *inter alia*, on the absence of viruses in the propagation stocks. The following steps are recommended to produce high quality material: (i) preparation of nuclear stocks from tissue culture; (ii) increase of virus-free nuclear stocks by rapid propagation (commercial laboratories); (iii) growing of foundation stocks under insect-proof conditions; and (iv) distancing propagation fields from commercial lily fields; these fields should be sprayed with insecticides and oils. At each step the material should be checked for viruses and the grower will choose the type of stock according to his needs. The stocks released to the grower should be of a quality that will enable him to grow them for at least three seasons. It is therefore advisable to grow new stocks far away from old infected ones. (*L*)

Virus Diseases in *Hippeastrum*

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The ornamentals industry is a major economic branch of modern agriculture. *Hippeastrum* (amaryllis) is cultivated for both cut flowers and pot plants, and is grown mainly in the Netherlands, Israel and South Africa. In 1993 the total value of *Hippeastrum* sold as cut flowers at the Dutch auctions was 27.5 million Dutch guilders and as pot plants, 4.3 million. In 1993, approximately one million bulbs at a value of U.S. $200,000 were exported from Israel to the United States. The export potential is two million dollars. The required product is *Hippeastrum* bulbs bigger than size 24, which will flower at Christmas time and develop two flowering stems.

One of the factors that limit the expansion of the growing area in Israel is virus contamination of the bulb stock. During surveys of *Hippeastrum* nurseries in Israel, three viruses were identified: Hippeastrum mosaic virus (HiMV), cucumber mosaic virus (CMV) and nerine latent virus (NeLV). The first and second viruses are transmitted in a non-persistent manner by the aphids *Myzus persicae* and *Aphis gossypii*; the vector of NeLV is unknown. Tomato spotted wilt virus (TSWV) and tobacco mosaic virus (TMV) have been detected occasionally in *Hippeastrum*.

The best strategy to minimize virus-induced crop losses in *Hippeastrum* is to use virus-tested propagation stock. Growing *Hippeastrum* in an insect-proof greenhouse with soil heating will

shorten the growing period, and greatly increase bulb size and flower yields. Diagnostic methods for viruses and certification schemes for *Hippeastrum* are being prepared for wider use. (*L*)

Identification of Leek Yellow Streak Virus (LYSV) in Different Garlic Species

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Garlic (*Allium sativum*), elephant garlic and great-headed garlic (*A. ampeloprasum*) are all solely vegetatively propagated. Therefore viral infestation is transmitted from one season to the next in the propagation material and all garlic in Israel harbors viruses. The most damaging virus in *A. sativum* is onion yellow dwarf virus (OYDV). Cultivars of *A. ampeloprasum* are infected with leek yellow streak virus (LYSV) or with both OYDV and LYSV. By screening introduction plots and collections of wild *Allium* species, we observed symptoms different from those typical to OYDV. No reaction was noticed when extracts from various cultivars of *A. ampeloprasum* were tested with specific OYDV antiserum. In addition, extracts from elephant garlic caused local lesions in *Chenopodium quinoa* and *C. amaranticolor*, whereas OYDV infects only garlic. The virus infecting test plants was isolated and used for eliciting specific rabbit antiserum. The specificity of the antiserum produced at Bet Dagan was similar to a serum obtained from Holland (IPO-DLO).

Almost all garlic grown in Israel is of the cultivar 'Shani', in which LYSV has not been detected. However, the possibility exists that OYDV confers cross protection and the introduction of virus-free garlic through meristem culture may render cv. Shani susceptible to LYSV and additional viruses introduced into Israel with bulbs for flower propagation. An identification and testing system for these viruses is essential if we are to be able to produce and maintain virus-free material. (*L*)

Studies of Grapevine Yellows in Israel – Occurrence, Identification and Spread

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Grapevine yellows diseases (YDs) were described for the first time in France in the 1950s. Since then the phenomenon has assumed various shapes and was reported in many countries and regions in a static or spreading manner. Flavescence dorée (FD) is one of the most studied YD of grapevine, caused by mycoplasm-like organisms (MLOs) and transmitted by propagation material and *Scaphoideus titanus* in an epidemic manner. Other YDs demonstrating similar symptoms have been spreading since the end of the 1980s in many grape-growing countries. In Israel, yellows symptoms were observed for the first time in the Jordan Valley on table grapes in the 1970s and the affected vineyards were uprooted. In 1987/88 a few Chardonnay grapes exhibiting yellows symptoms were spotted in the Golan Heights, and since then the disease has spread to other geographic regions and varieties.

Disease symptoms are as follows: growth delay in spring, sectorial or extensive yellowing (or reddening) of the leaves, downrolling of the blades, partial necrosis of main veins and blades, incomplete ripening of the wood, pustule formation on the canes, dying of the inflorescence, and fruit shrinkage.

Serological tests (ELISA) carried out with FD antiserum did not produce a positive reaction, suggesting the lack of a serological relationship between FD-MLO and the Israeli YD. Further analysis in a procedure using polymerase chain reaction primers derived from 16s rRNA gene fragments of MLOs showed a positive reaction for the presence of non-FD-MLOs with a constant restriction profile.

A few leafhoppers, potentially YD vectors, were trapped in the Golan Heights vineyards this year.

Further investigations aiming at molecular diagnosis of the causal agent, trapping of potential leafhopper vectors, and transmission trials will be conducted in the coming years. (*L*)

Cucurbit Yellowing Virus (CYV), A New Virus Disease of Melon and Cucumber

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Cucumber (*Cucumis sativus*) and melon (*Cucumis melo*) crops grown in Israel have shown severe leaf yellowing during the last few years. It was suggested that the phenomenon is due to mineral deficiencies or to a physiological plant response to toxins released by *Bemisia tabaci* during feeding. Recently we have found that the disease is caused by a whitefly-transmitted virus, designated tentatively cucurbit yellowing virus (CYV). In transmission experiments with *B. tabaci*, the disease was transmissible from field-collected plants to healthy melon, watermelon (*Cucumis lanatus*) and cucumber test plants grown in an insect-proof greenhouse. The virus is not transmissible by mechanical inoculation, and has a long incubation period (4 weeks). Symptoms are visible first on the older leaves. Two dsRNA fractions (2.5 kB and 17 kB) were detected in infected plants by polyacrylamide gel electrophoresis.

Semipurified viral preparations obtained from plants that had been inoculated in the greenhouse contained closterovirus-like particles 800–1000 nm in size.

The effect of the disease on melon yield was estimated as follows: Melon seedlings (var. 'Arava') were inoculated by whiteflies; the insects had acquired the virus from CYV greenhouse-infected source plants during a 48 h acquisition access followed by a 48 h inoculation feeding on the target plants. The inoculated melon plants and an uninoculated control group were transferred to a greenhouse protected by 50-mesh screens. The yield (export quality) obtained from the inoculated plants was 50% lower than that of the healthy control. (*P*)

Long-Distance Movement of Tobamovirus Hybrids in Tobacco Plants

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The tobamoviruses tobacco mosaic virus (TMV) and odontoglossum ringspot virus (ORSV), differ in the range of plant species that each can infect systemically. Both viruses similarly infect systemically *Nicotiana benthamiana*, but differ in the ability to infect systemically *N. tabacum*. Systemic infection of *N. tabacum* by TMV occurs rapidly, resulting in mosaic symptoms and considerable reduction in plant growth. In contrast, ORSV is confined almost completely to the inoculated leaves, with no resulting symptoms or effects upon their growth. A TMV chimera expressing the ORSV capsid protein gene, spread cell-to-cell similarly to TMV, but was deficient in long-distance movement and systemic infection.

The role of coat protein sequences in phloem-mediated, long-distance movement was studied by creating mutants and hybrids of TMV and TMV-CP-O. The variable region located on the outside of virions was exchanged between TMV and TMV-CP-O using polymerase chain reaction site-directed mutagenesis. Large blocks of coat protein sequences were exchanged between restriction endonuclease sites engineered into TMV and TMV-CP-O. A *Pac*I site is present in the coat protein gene (nt 5784). The corresponding *Pac*I site in TMV-CP-O was introduced at nt 5787. *Age*I sites were introduced into TMV and TMV-CP-O at nts 6120 and 6135, respectively. This allows cutting the coat protein genes into three units. All combinations of these three units were made. The resulting mutants were tested for rapid long-distance movement in tobacco. (*L*)

A Defective Movement Protein of TMV in Transgenic Plants Confers Resistance to Multiple Viruses, Whereas the Functional Analog Increases Susceptibility

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Transgenic tobacco plants that express a gene encoding a defective mutant of the tobacco mosaic virus (TMV) movement protein, which are known to be resistant to several tobamoviruses [Lapidot *et al.*, *Plant J.* 4:959-970 (1993)], were inoculated with viruses from different taxonomic groups to determine the extent of resistance. There were significant delays in the time of appearance of disease symptoms and/or there was reduced systemic accumulation of virus in upper leaves of plants inoculated with tobacco rattle tobravirus, peanut chlorotic streak caulimovirus, alfalfa mosaic ilarvirus, tobacco ringspot nepovirus, cucumber mosaic cucumovirus and tomato spotted wilt tospovirus. Conversely, tobacco plants that express a gene encoding the functional TMV wild-type movement protein accelerated symptom development, enhanced the severity of symptom formation, and/or increased the accumulation of these viruses and, additionally, TMV. Our results indicate that there are similar functions among the movement proteins of a number of plant viruses despite the apparent lack of sequence similarity between them. Moreover, the results demonstrate a unique type of engineered virus resistance that is effective against pathogens in a wide range of plant virus groups. (*L*)